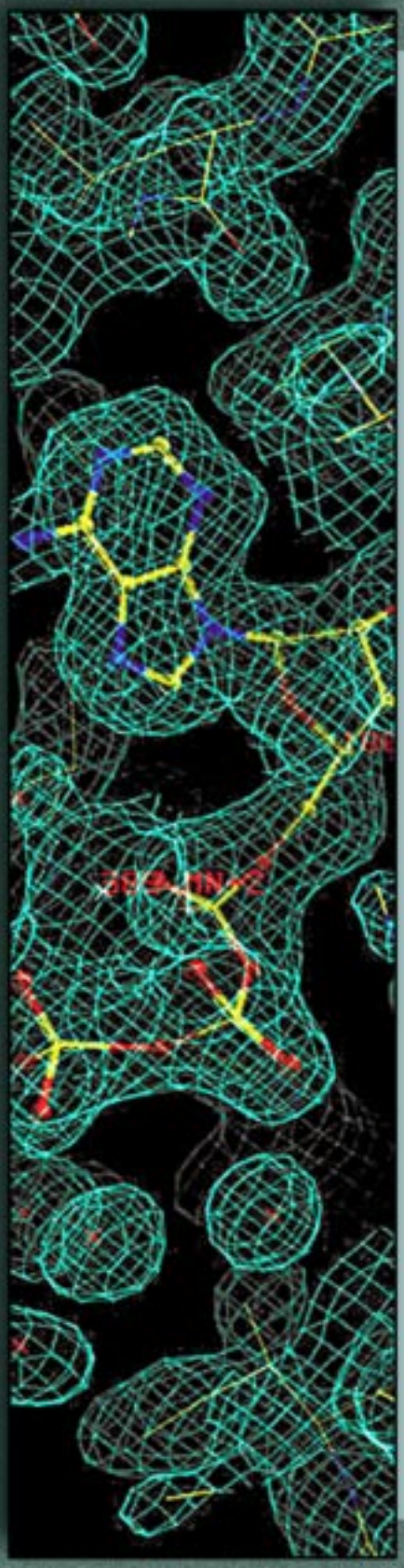
# World Class Protein Crystallography



*The*

# Macromolecular Crystallography Facility

*at the*

# Advanced Light Source

BERKELEY LAB

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BERKELEY NATIONAL LABORATORY

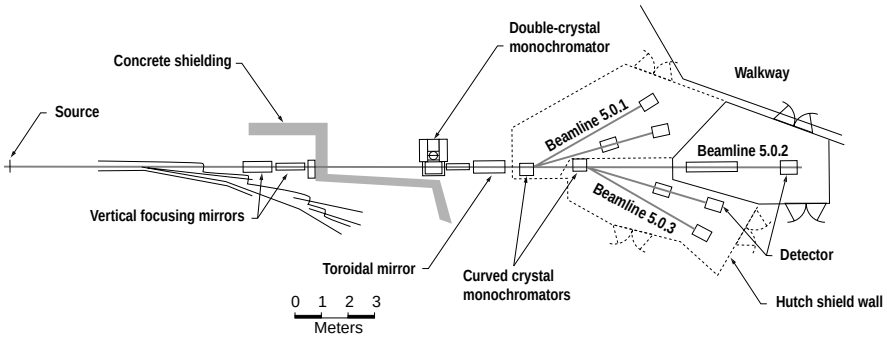
# The Macromolecular Crystallography Facility

Funded principally by the Office of Biological and Environmental Research of the U. S. Department of Energy, the Macromolecular Crystallography Facility at the Advanced Light Source (MCF/ALS) is a "national user facility" open to scientists from academic, industrial, and government laboratories. Operated by the Physical BioSciences Division of the Lawrence Berkeley National Laboratory (Berkeley Lab), the MCF offers a complete spectrum of protein-crystallography capabilities. Since the beginning of MCF operation for crystallography in November 1997, scientists have successfully collected multiple-wavelength anomalous diffraction (MAD) data, diffraction data from microcrystals, and "conventional" diffraction data with extremely rapid throughtput. By February 1999, more than 200 users from 52 research groups had visited the MCF.

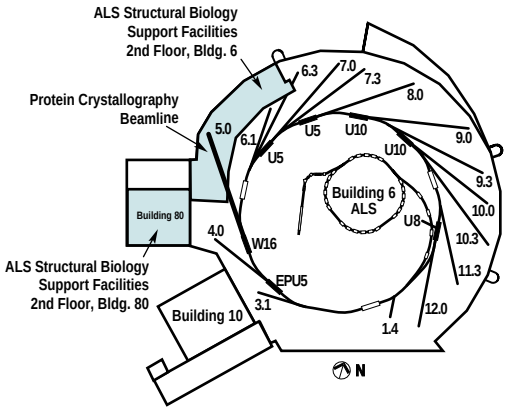
The heart of the MCF comprises beamlines and crystallography stations at the ALS. The x-ray source for the MCF is a high-field, multipole wiggler that can illuminate up to three semi-automated crystallography beamlines for simultaneous use. Receiving the on-axis, brightest portion of the wiggler light, the central beamline (Beamline 5.0.2) operates over the wavelength range from 0.9 Å to 4.0 Å. Optimized for MAD methods, it is equally suitable for monochromatic crystallography. The principal components of the beamline include a front end, vertically collimating premirror, double-crystal silicon (111) monochromator with a fixed-height exit beam, toroidal focusing mirror, kappa-axis goniometer, and a CCD-based detector (an ADSC Quantum-4 2 × 2 array) for fast readout.

Two additional fixed-wavelength beamlines for monochromatic crystallography at 1.0 Å (Beamlines 5.0.1 and 5.0.3) are under development for operation beginning in January 2000. Like the central station, the side stations will have optics that are self-aligning onto the sample collimator and will offer cryo-cooled sample environments.

Looking farther ahead, a program is now under way with a commercial supplier to develop superconducting bend magnets (superbends) to replace three of the 36 dipole magnets in the ALS storage ring. The 5-T magnetic field of the proposed superbends will increase the brightness and flux at 1 Å by a factor of 10 over the bend magnets now in place. In collaboration with the ALS, the MCF is planning to develop three more beamlines for MAD crystallography around a superbend in Sector 4 adjacent to the Beamline 5.0 complex.




**Layout showing present and future MCF beamlines. Beamline 5.0.2 is operational. Beamlines 5.0.1 and 5.0.3 are under development.**




**Plan view of the ALS and adjacent buildings shows Beamline 5.0 at the MCF and the Structural Biology Support Facilities.**

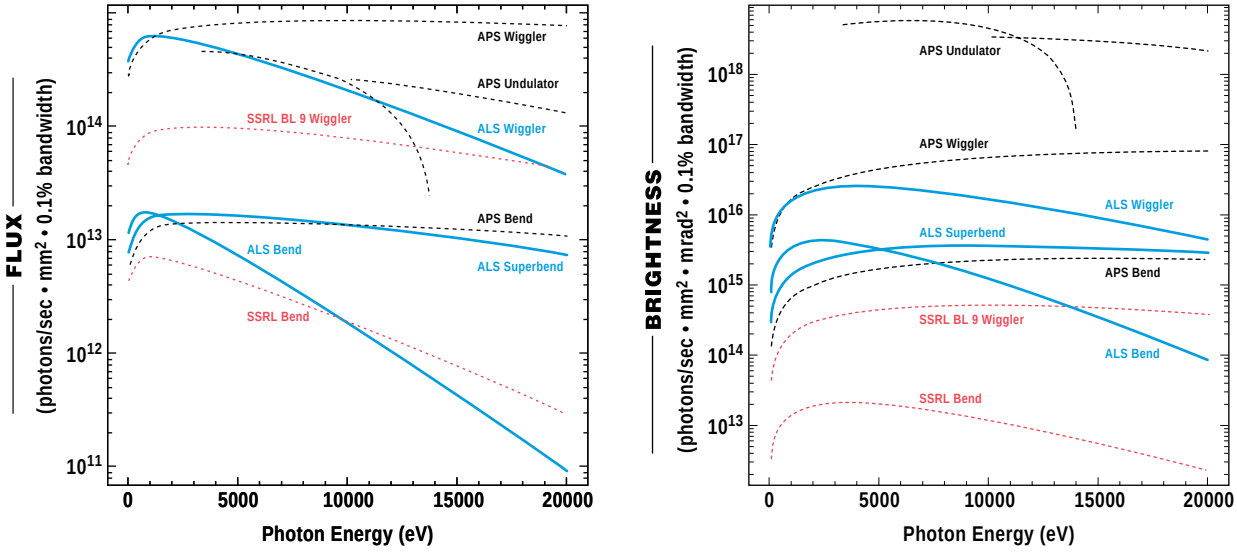
Users of the MCF have access to the Structural Biology Support Facilities. On the second floor of the ALS building directly over the protein-crystallography stations and on the second floor of contiguous Building 80, these facilities provide laboratories and highly automated instrumentation for the preparation and characterization of samples by a variety of techniques, including microscopy and spectroscopy. They also have high-performance computer workstations with graphics capabilities that are networked to beamline computers for on-site data analysis. Scientific and technical staff at the MCF are available to support visiting crystallographers in every phase of their stay. ■

# A Performance that Stands with the Best

Operating at a beam energy of 1.9 GeV, the ALS is an extremely good source for the intermediate-energy x rays used in protein crystallography for several reasons: (1) the ALS has a state-of-the-art, third-generation storage ring with a low emittance (product of electron beam size and angular divergence); (2) the tiny vertical beam size allows the wiggler gap to be set to a small value (14 mm in Beamline 5.0.2) resulting in a high magnetic field and thereby a high flux at short wavelengths; and (3) the ALS runs at a high current of 400 mA, further boosting the flux.

Together these factors result in the high flux and angular collimation on the sample required for advanced crystallography experiments in frontier areas, such as ultrahigh resolution (1 Å or better), structural determination from microcrystals (e.g., membrane proteins), studies of large macromolecular complexes (e.g., ribosomes and multiprotein complexes), and determination of large numbers of structures in coordinated projects (e.g., structural genomics and iterative structure-based drug design). In addition, the beam is positionally very stable and the storage ring operates with high reliability (above 95% availability), thereby allowing the crystallographer to take advantage of the wiggler performance for both advanced and more routine crystallography.

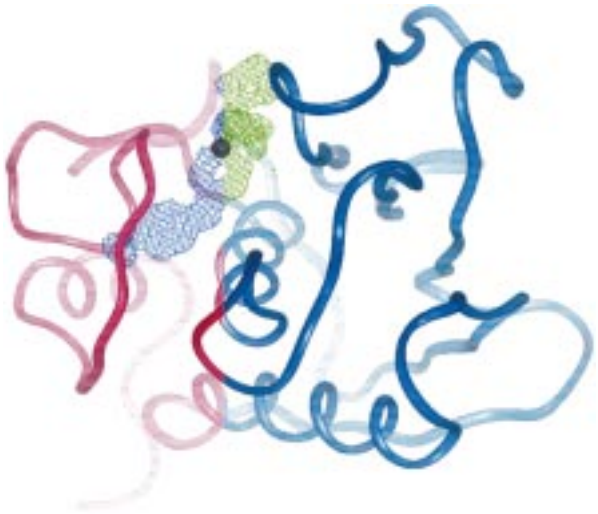
While flux (photons/sec) and brightness (flux per unit phase-space area of the radiation source, i.e., flux per unit source area per unit solid angle of the radiation cone) are the most commonly cited measures of performance for a synchrotron-radiation source, the flux into the phase-space acceptance of the sample is what determines the count rate and resolution in an actual experiment. For protein crystals, the acceptance is defined by the crystal size (or its mosaicity) and the angular separation required for adequate resolution of diffracted beams. For most synchrotron sources, the vertical source is small enough that it can be focused even onto microcrystals while retaining the required angular collimation. In most practical cases of protein crystallography, therefore, the figure of merit for an x-ray source that determines the maximum attainable flux into the sample acceptance is the horizontal brightness; that is, the horizontal angular flux density divided by the horizontal source size. Based on this criterion, the ALS is a world-class source for protein crystallography, a conclusion now being proven in the daily experience of the growing number of MCF users. Moreover, radiation damage limits the maximum integrated intensity a protein crystal can tolerate before high-resolution information is lost. In many cases, ALS wiggler Beamline 5.0.2 reaches this fundamental limit in less than four hours of accumulated exposure. ■




**Comparison of the flux (left) and brightness (right) of representative existing sources at the Advanced Photon Source (APS) and the SPEAR2 storage ring at the Stanford Synchrotron Radiation Laboratory (SSRL) with the performances of the wiggler, proposed superbends, and bend magnets at the ALS shows that at the MCF is competitive with existing facilities for protein crystallography.**

# Demonstrated High-Resolution Microcrystal Capability

Collecting data from microcrystals provides a good test of performance, and the MCF is getting surpassing grades. Data collection from microcrystals requires a bright synchrotron source in order to simultaneously focus sufficient flux onto the crystal and obtain high angular resolution of the diffracted beams. It is hard to overemphasize the significance of enhanced capabilities for microcrystal experiments or to accurately predict the impact of this technological advance. At a minimum, the successful routine determination of structures from microcrystals would dramatically increase the number of macromolecules available for crystallographic study.



**Structure of collagenase complexed with an inhibitor determined from data obtained to a resolution of 1.8 Å at the MCF from a frozen microcrystal with dimensions of a few tens of microns. [Data courtesy of M. Browner and B. Lovejoy, Roche Bioscience.]**

Producing suitable crystals is now the major hurdle in crystallography, and a common problem is that initial biomolecule crystals are too small for analysis. Since the standard approaches to increase crystal size can introduce extended delays, with uncertain prospects for breakthroughs, some classes of experiments will become feasible only with the ability to analyze microcrystals. Screening initial microcrystals for diffraction also offers a new approach for identifying the most promising crystallization conditions.

Membrane proteins are a classic example of molecules difficult to crystallize. Microcrystals of membrane proteins are typical, and large, well-ordered crystals are very rare. Analysis of membrane proteins, including the ABC transporters that play large roles in disease and drug resistance, will reveal a new world of channels, pumps, receptors, pores, and antigens. An expanded database of membrane-protein structures will facilitate prediction, design, and drug discovery.

In iterative structure-based drug design, real-time feedback of structural information for a given molecule is used to direct subsequent structural modifications as part of, say, pharmaceutical development, and to influence the priorities with which subsequent structures are examined. This is a particular example of a more general class of problems in which microcrystals are essential in investigating the interactions of proteins with small molecules, such as substrates, inhibitors, and drugs. Ligands often cause conformational changes that may destroy large crystals but leave microcrystals ordered. Binding sites are also saturated more quickly in microcrystals, allowing transient bound states to be imaged after flash freezing. These studies will help reveal short-lived functional states and critical conformational transitions.

In a striking example, scientists from Roche Bioscience gathered an entire data set from a frozen microcrystal of collagenase complexed with an inhibitor to a resolution of 1.8 Å and completed the structure determination at the MCF. They have also completed to high resolution the structures of several other collagenase/inhibitor complexes using data from microcrystals. These complexes are a part of the Roche structure-based drug design program for inflammatory diseases. Equally striking, the structure of the transmembrane proton pump, bacteriorhodopsin, from the purple membrane of *Halobacterium halobium* has been solved to 1.55 Å from data collected from microcrystals with dimensions about $70 \times 70 \times 20\ \mu m^3$ by the groups of Hartmut Luecke and Janos Lanyi (University of California, Irvine). This is the highest resolution structure to date for a membrane protein and the feat is even more impressive because of the small size of the crystals. ■

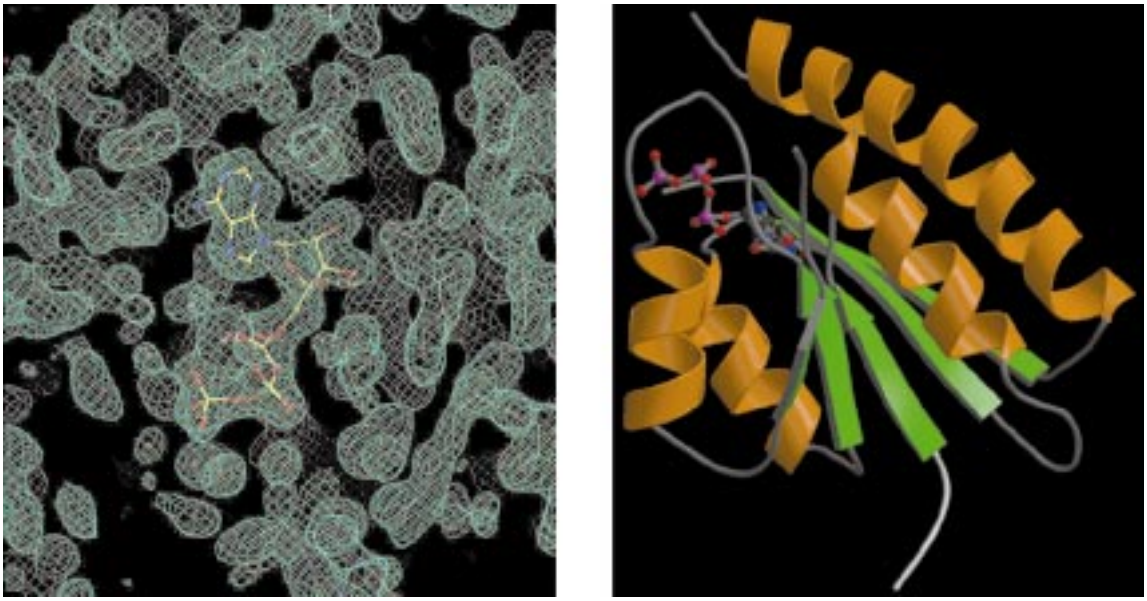
# High Throughput for Routine and Large-Scale Projects

Simultaneously providing accuracy and high throughput is a challenge that is being met at the MCF. Although speed and accuracy are always desirable even for routine projects, large-scale projects pose special needs, including use of multiple-wavelength anomalous diffraction (MAD) methods to obtain phase information on large numbers of samples. Structural genomics, whose goal is to characterize a significant fraction of the proteins coded by an entire genome, is a current example of a large-scale, coordinated project.

To test the feasibility of structural genomics, Sung-Hou Kim (University of California, Berkeley, and Berkeley Lab) and his colleagues are using the MCF in a pilot study of the fully sequenced model thermophilic archaebacterium, *Methanococcus jannaschii*. The Kim group has chosen several gene products from this organism—some with known homologues and some without—and have begun to determine their structures.

The goal of this project is to determine the structures of the approximately 1800 gene products expressed in the microbe. In particular, the protein-folding pattern provides an important insight into the biochemical function of the gene product. The expected results of the project include an expanded basis set of folds for structure prediction, a feast of new structures for functional analysis, and improved methods for rapid x-ray structure determination. The principal focus on new folds makes MAD analysis a necessity because models for molecular replacement are not available.

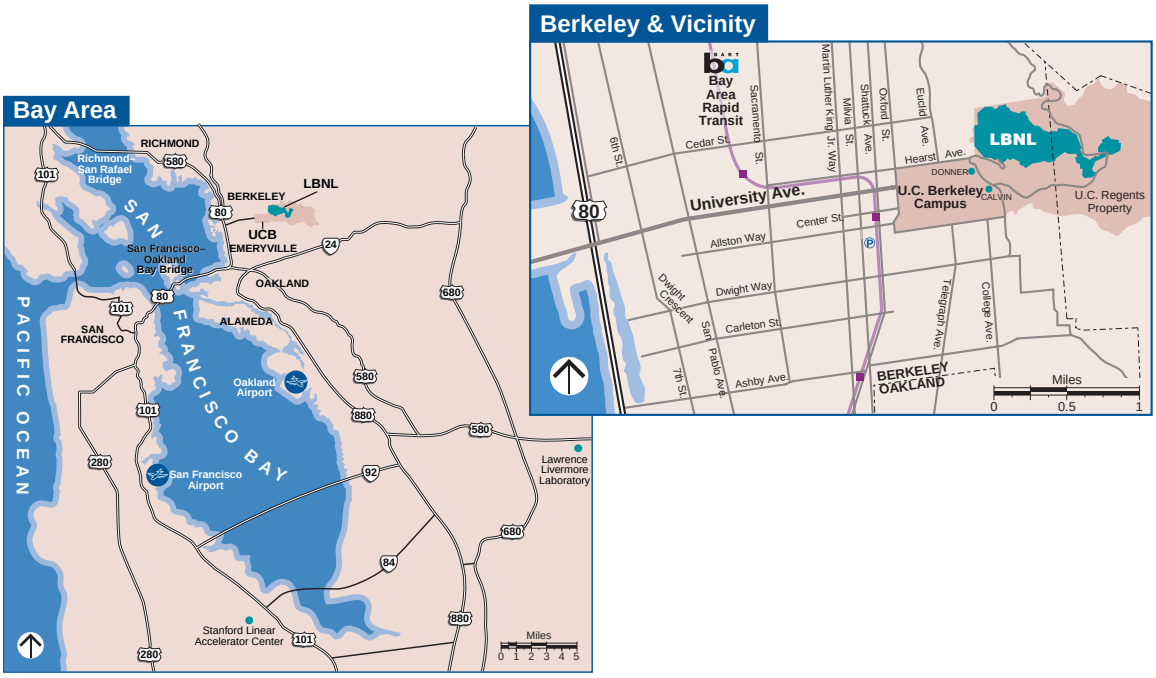
Early results have already allowed the roles of two "hypothetical" proteins to be tentatively identified from their structures alone. With data gathered at the MCF, the Kim group has determined the structures of two hypothetical proteins, MJ0577 and MJ0882 from *M. jannaschii*. Both structures were solved and refined within a few days after data collection was completed. The set of high-quality experimental phases from MAD measurements at Beamline 5.0.2 have proven to be the key factor for interpreting and modeling the structures of the protein and ligands. In both cases, the group obtained important information based on structural similarities to other proteins. For example, MJ0577 was identified as an ATP-binding protein after examination of the electron density map showed bound ATP. The discovery of the ATP immediately narrows down the possible biochemical functions of this hypothetical protein. ■



**Structure of hypothetical protein MJ0577 in the hypothermophile *Methanococcus jannaschii*. (left) Electron-density map derived from MAD experimental phases clearly shows a bound ATP. (right)The tertiary structure of MJ0577 is a nucleotide binding fold. [Data courtesy of L.-W. Hung, T. Zarembinski, J. Mueller-Dieckmann, and S.-H. Kim, University of California, Berkeley, and Berkeley Lab.]**

## Getting to the MCF/ALS

The Berkeley Lab is located on a site in the hills directly above the campus of the University of California, Berkeley, and is readily accessible by automobile from anywhere in the San Francisco Bay Area and by limousine or taxi from the San Francisco and Oakland airports. The Bay Area Rapid Transit (BART) system also provides convenient access via its station in downtown Berkeley. Berkeley Lab operates weekday shuttle services: an off-site shuttle between locations around the UC campus and downtown Berkeley to the laboratory and an on-site shuttle. The MCF is located in Building 6 (the ALS building) with the crystallography station on the experimental floor and offices upstairs.




## For More Information

Operating as a national user facility, the MCF is open around the year to scientists from academic, industrial, and government laboratories, who may work in collaboration with MCF scientific staff or submit proposals that will be peer-reviewed as part of the Independent Investigator program at the ALS. Beam time is free to those conducting nonproprietary research; there is a modest charge to cover operating costs for proprietary usage. A Web-based application and review system is under development. Information about the MCF scientific program, about the beamline at the ALS, and about submitting a proposal is available from the persons listed below. Information about the ALS and its beamlines, about the user program, and about proposals is also available on the Web at URL: www-als.lbl.gov/als/.

| MCF Spokesperson | Beamline Contact | Proposal Forms |
|---|---|---|
| Thomas N. Earnest<br>Physical BioSciences Division<br>Berkeley Lab, MS 6-2100<br>Berkeley, CA 94720 | Carl Cork<br>Physical BioSciences Division<br>Berkeley Lab, MS 6-2100<br>Berkeley, CA 94720 | Ruth Pepe<br>User Services Administrator<br>Advanced Light Source<br>Berkeley Lab, MS 6-2100<br>Berkeley, CA 94720 |
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